DRAFT REPORT

Study Title

BOVINE CORNEAL OPACITY AND PERMEABILITY ASSAY WITH TWO TIME EXPOSURES AND HISTOLOGY

Product Identity

Z
AA

Author

Janet Luczak, M.G.A.
Toxicologist

Study Completion Date

Pending Final Report

Testing Facility

Institute for In Vitro Sciences, Inc.
21 Firstfield Road, Suite 220
Gaithersburg, MD 20878

Laboratory Project Number

06AG29-AG30.350025

Laboratory Project ID

4560

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA section 10 (d) (1) (A), (B), or (C).

Company:

Company Agent: Date: _____

Signature: _____

Title

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study meets the requirement of 40 CFR § 160 with the following exceptions:

The identity, strength, purity, composition, and stability or other characteristics to define the test substances have not been determined by the testing facility.

The stability of the test substances has not been determined by the testing facility under the test conditions and is not included in the final report.

Submitter:

_____ Date:_____

Sponsor:

_____ Date:_____

Study Director:

_____ Date:_____

Janet Luczak, M.G.A.
Toxicologist

TABLE OF CONTENTS

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS 2

STATEMENT OF COMPLIANCE 3

TABLE OF CONTENTS 4

QUALITY ASSURANCE STATEMENT 5

STUDY PERSONNEL 6

STUDY REPORT 7

STUDY MATERIALS 8

TEST METHOD 8

PROTOCOL CHANGES 10

CONTROLS 10

STUDY ACCECPTANCE CRITERIA 10

DATA ANALYSIS 11

STUDY RETENTION 11

STUDY RESULTS AND DISCUSSION 12

STUDY CONCLUSION 15

APPENDIX A
- SP350025 (PROTOCOL) 18
- PROTOCOL ATTACHMENT 1 26

APPENDIX B (RAW DATA) 28

APPENDIX C (CERTIFICATE OF ANALYSIS) 32

APPENDIX D (COPY OF THE HISTOPATHOLOGY REPORT) 35

QUALITY ASSURANCE STATEMENT

Study Title: Bovine Corneal Opacity and Permeability Assay with Two Time Exposures and Histology

Study Number: 06AG29-AG30.350025

Study Director: Janet Luczak, M.G.A.

This study has been divided into a series of in-process phases. Using a random sampling approach, Quality Assurance monitors each of these phases over a series of studies. Procedures, documentation, equipment records, etc., are examined in order to assure that the study is performed in accordance with the U.S. EPA GLP Standards (40 CFR 160) and to assure that the study is conducted according to the protocol and relevant Standard Operating Procedures.

The following are the inspection dates, phases inspected and report dates of QA inspections of this study:

Phase Inspected	Audit Date(s)	Reported to Study Director	Reported to Management
Protocol and Initial Paperwork			
Draft Report and Data			
Final Report			

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

_____ _____

Amanda K. Ulrey, RQAP-GLP Date
Quality Assurance

STUDY PERSONNEL

BOVINE CORNEAL OPACITY AND PERMEABILITY ASSAY WITH TWO TIME EXPOSURES AND HISTOLOGY

Study Director:

_____ _____
Janet Luczak, M.G.A. Date
Toxicologist

Laboratory Personnel:

Principle Investigator: (Histopathology)	Stewart B. Jacobson, DVM, DACVP Charles River Laboratories, Pathology Associates (PAI)-Maryland
Laboratory Supervisor:	Gregory Moyer, M.B.A.
Primary Technical Staff:	Nathan Wilt, B.S. Allison Hilberer, B.S. Valerie Deoudes, B.S. Nicole Barnes, B.S. Jennifer Nash, M.S.

.

STUDY REPORT

Study Title: Bovine Opacity and Permeability Assay with Two Time Exposures and Histology

Sponsor:

Test Facility: Institute for In Vitro Sciences, Inc.
21 Firstfield Road, Suite 220
Gaithersburg, MD 20878

Test Substance Identification

IIVS Test/Reference Substance Number	Sponsor's Designation	Physical Description	Expiration Date	Receipt Date	Storage Conditions*
06AG29	Z	clear light brown non-viscous liquid	7/05/07	8/10/06	room temperature
06AG30	AA	clear colorless non-viscous liquid	2/22/07	8/10/06	room temperature

* Protected from exposure to light

Study Initiation Date: August 14, 2006

Experimental Start Date: August 15, 2006

Experimental End Date: (Pending Histopathology Report)

Study Completion Date: (Pending Final Report)

Study Objective: To evaluate the potential ocular irritancy/toxicity of the test substance using the Bovine Corneal Opacity and Permeability Assay (BCOP)

Test Method: Bovine Corneal Opacity and Permeability Assay (BCOP)

Test System: Bovine Corneas

STUDY MATERIALS

Media and Reagents:	Minimum Essential Media (EMEM) without phenol red containing 1% FBS and 2 mM L-glutamine (Complete MEM)
	Minimum Essential Media (EMEM) with phenol red containing 1% FBS and 2 mM L-glutamine (Complete MEM) (used for rinsing only)
	Hanks' Balanced Salt Solution (HBSS) including Ca^{++}, Mg++, and containing Pen/Strep
	Fluorescein (4 mg/mL)
	10% Formalin (Fixative)
Equipment:	Opacitometer
	Molecular Devices Vmax Kinetic Microplate Reader
	Corneal Holders
	32^{o}C Incubator
	Micropipettors(calibrated)

TEST METHOD

Preparation of Test Substance

As instructed by the Sponsor, each test substance was administered to the test system without dilution. The pH of each of the test substances was determined using pH paper (EMD Chemicals Inc.). Initially, each of the test substances was added to 0-14 pH paper with 1.0 pH unit increments to approximate a narrow pH range. Next, the test substance, Z was added to 0-6 pH paper with 0.5 pH unit increments and AA was added to 5- 10 pH paper with 0.5 pH unit increments, to obtain a more precise pH value. The pH values obtained from the narrower range pH paper are recorded in Table 1.

Preparation of the Test System

Bovine eyes were obtained from a local abattoir as a by-product from freshly slaughtered animals (J.W. Trueth & Sons, Inc., Baltimore, MD). The eyes were excised and then placed in Hanks' Balanced Salt Solution, containing Penicillin/Streptomycin (HBSS), and transported to

the laboratory on ice packs. Immediately upon receipt of the eyes into the laboratory, preparation of the corneas was initiated.

The eyes were grossly examined for damage and those exhibiting defects were discarded. The tissue surrounding the eyeball was carefully pulled away and the cornea was excised such that a 2 to 3 mm rim of sclera was present around the cornea. The isolated corneas were then stored in a petri dish containing HBSS until they were mounted in a corneal holder. The corneas were mounted in the holders with the endothelial side against the O-ring of the posterior chamber. The anterior chamber was then positioned on top of the cornea and the screws were tightened. Starting with the posterior chamber, the two chambers were then filled with Minimum Essential Medium (EMEM) without phenol red, containing 1% fetal bovine serum and 2mM L-glutamine (Complete MEM). Each corneal holder was uniquely identified with a number written in permanent marker, on both the anterior and posterior chambers. The corneal holders were incubated at 32 ± 1ºC for a minimum of 1 hour.

Bovine Corneal Opacity and Permeability Assay

After a minimum of 1 hour of incubation, the corneas were removed from the incubator. The medium was removed from both chambers and replaced with fresh Complete MEM. The initial opacity was determined for each cornea using a Spectro Designs OP-KIT opacitometer. Three corneas, whose initial opacity readings were close to the median opacity for all the corneas, were selected as the negative control corneas. The treatment of each cornea was identified with the test substance number written in permanent marker on colored tape, affixed to each holder. The medium was then removed from the anterior chamber and replaced with the test substance, positive control, or negative control.

The liquid test substances, Z and AA were tested neat. An aliquot of 750 µl of the test substance, positive control, or negative control was introduced into the anterior chamber while slightly rotating the holder to ensure uniform distribution over the cornea. Each treated cornea was completely covered with the test substance. One group of three corneas was incubated in the presence of the test substances at 32 ± 1ºC for 3 minutes. A second group of three corneas was incubated in the presence of the test substances at 32 ± 1ºC for 10 minutes. Three corneas were incubated in the presence of the positive control substance at 32 ± 1ºC for 10 minutes. Two corneas were incubated in the present of the negative control at 32 ± 1ºC for 60 minutes. After the 3 , 10 and 60-minute exposure times, the control or test substance treatments were removed. The epithelial side of the corneas was washed at least three times with Complete MEM (containing phenol red) to ensure total removal of the control or test substance. The corneas were then given a final rinse with Complete MEM (without phenol red). The anterior chamber was refilled with fresh Complete MEM and an opacity measurement was performed. The corneas exposed to the test or positive control substance were returned to the incubator for approximately 2 hours. The corneas exposed to the negative control (60 minutes exposure) were returned to the incubator for approximately 1 hour. After each designated post-exposure period, a final measure of opacity was obtained.

After the final opacity measurement was performed, the medium was removed from both chambers of the holder. The posterior chamber was filled with fresh Complete MEM and 1 mL of a 4 mg/mL fluorescein solution was added to the anterior chamber. The corneas were then incubated in a horizontal position (anterior side up) for approximately 90 minutes at 32 ± 1ºC. At the end of the 90-minute incubation period, the medium was removed from the posterior chamber and placed into tubes numbered corresponding to chamber number. Aliquots of 360 µL from the numbered tubes were placed into their designated wells on a 96-well plate. The optical density at 490 nm (OD_{490}) was determined using a Molecular Devices *V*max kinetic microplate reader. If the OD_{490} value of a control or test substance sample was 1.500 or above, a 1:5 dilution of the sample was prepared in Complete MEM (to bring the OD_{490} value within the linear range of the platereader). A 360 µL sample of each 1:5 dilution was transferred to its specified well on the 96-well plate. The plate was read again and the final reading was saved to a designated print file.

After the medium was removed for the permeability determination, each cornea was carefully separated from its corneal holder and transferred to an individual prelabeled tissue cassette containing a biopsy sponge. The endothelial surface of each cornea was placed on the sponge to protect it. The cassettes were placed in 10% neutral buffered formalin to fix the corneal tissue for at least 24 hours.

The fixed corneas were transferred to Charles River Laboratories, Pathology Associates (PAI)-Maryland for embedding, sectioning and staining. Each cornea was paraffin-embedded, bisected, and the two halves mounted in the paraffin block so that a section of each half could be cut and placed on a single slide. Each slide was then stained with hematoxylin and eosin. Slides were returned to IIVS for evaluation.

PROTOCOL CHANGES

Protocol Amendments	None

CONTROLS

Positive Control:	Ethanol (Pharmaco, 200 proof, USP) (tested without dilution)
Negative Control:	Sterile, Deionized Water (Quality Biological)

STUDY ACCEPTANCE CRITERIA

The BCOP assay was accepted when the positive control (ethanol) caused an *in vitro* score that was within two standard deviations of the historical mean.

DATA ANALYSIS

Opacity Measurement: The change in opacity for each cornea (including the negative control corneas) was calculated by subtracting the initial opacity reading from the final opacity reading. These values were then corrected by subtracting from each the average change in opacity observed for the negative control corneas. The mean opacity value of each treatment group was calculated by averaging the corrected opacity values of each cornea for that treatment condition.

Permeability Measurement: The mean OD_{490} for the blank wells was calculated. The mean blank OD_{490} was then subtracted from the raw OD_{490} of each well (corrected OD_{490}). Any dilutions that were made to bring the OD_{490} readings into the linear range of the platereader (OD_{490} should be less than 1.500), had each diluted OD_{490} reading multiplied by the dilution factor. The final corrected OD_{490} of each treatment group and the positive control was then calculated by subtracting the average corrected OD_{490} of the negative control corneas from the corrected OD_{490} value of each treated cornea:

Final Corrected OD_{490} = (raw OD_{490} – mean blank OD_{490}) – average corrected negative control OD_{490}

The mean OD_{490} value of each treatment group was calculated by averaging the final corrected OD_{490} values of the treated corneas for that treatment condition.

The following formula was used to determine the *in vitro* score:

In Vitro Score = Mean Opacity Value + (15 x Mean OD_{490} Value)

STUDY RETENTION

Upon completion of the final report, all raw data, copies of the reports, slides and tissue blocks will be maintained by the Institute for In Vitro Sciences, Inc.

STUDY RESULTS AND DISCUSSION

Opacity and Permeability Results

Table 1 summarizes the opacity, permeability, and *in vitro* score for the test substance or reference substance at each exposure time. Table 2 summarizes the opacity, permeability, and *in vitro* score for the positive control. Since the results of the positive control fell within two standard deviations of the historical mean (within a range of 39.4 to 64.2), the assay was considered valid. The opacity and permeability data for the individual corneas may be found in Appendix B.

Table 1
BCOP Results of the Test and Reference Substance

Assay Date	IIVS Test/Reference Substance Number	Sponsor's Designation	Conc.	Exposure Time	Mean Opacity Value	Mean OD_{490} Value	*In Vitro* Score	pH
8/15/06	06AG29	Z	Neat	3 minutes	4.3	0.565	12.8	3.5
				10 minutes	7.0	1.642	31.6	
	06AG30	AA	Neat	3 minutes	22.7	1.206	40.8	6.5
				10 minutes	44.7	2.025	75.0	

Table 2
BCOP Results of the Positive Control

Assay Date	Positive Control	Exposure Time	Mean Opacity Value	Mean OD_{490} Value	*In Vitro* Score
8/15/06	Ethanol	10 minutes	29.7	1.163	47.1

Histological Evaluation

The negative control treated corneal epithelium was composed of three layers. The basal cell layer was a well-formed, columnar-cell region directly attached to the Bowman's Layer. The basal cells were always tightly attached to each other. Several layers of wing cells covered the columnar basal layer. In both of these layers, the cell nuclei showed diffuse chromatin without clear nucleoli. Asymmetric clear spaces ("nuclear halos") were occasionally observed around nuclei in the wing and basal cell layers. These were an artifact of tissue processing and unrelated to treatment. Rare mitotic figures were seen in the basal layer. The squamous layer was flattened with limited cytoplasm and highly condensed nuclei. The stromal elements in the negative control treated corneas showed well-organized collagen matrix fibers with dispersed keratocytes. Keratocyte nuclei showed a range of morphologies from moderate sized (smaller than the epithelial nuclei) with diffuse basophilic staining, to narrow, elongated and condensed with dark basophilic staining. Cytoplasmic staining, when it was visible, was moderately basophilic. Rarely, cells with eosinophilic cytoplasmic staining were observed. Collagen bundles were generally parallel and well ordered. The Descemet's Membrane was prominent. The endothelial layer could be seen in most sections and was reasonably well maintained.

The positive control treated corneas showed the classic pattern of squamous layer coagulation (darkening) and marked vacuolization in the wing and basal cell nuclei. The loss of adhesion between the basal cells (cell to cell) and the basal lamina was marked. The epithelium was probably not viable at the time of fixation. Overall, the positive control-treated corneas were thicker than the negative control-treated corneas. In the stroma directly below Bowman's Layer, the collagen matrix showed slight hypereosinophilic staining suggestive of some coagulation. Below this zone, moderate/marked collagen matrix expansion extended past 50% depth. In the upper stroma, there was a decrease in the density of viable keratocytes as reflected by a marked increase in the frequency of keratocytes showing nuclear vacuolization. In the stroma below mid depth, the keratocytes showed a moderate increase in the frequency of cells with enlarged nuclei and cytoplasmic eosinophilia. The endothelial cells were generally intact (similar to negative control treated corneas).

Corneas treated with the test substance, Z, for three minutes exhibited diffuse loss of the squamous layer and blanching of exposed superficial wing cells. Multifocally, the cytoplasm of deeper wing and basal cells were finely vacuolated. The stroma was similar in thickness to the negative controls. Immediately subjacent to the epithelium there was minimal expansion of the collagen matrix and scattered keratocytes with eosinophilic cytoplasm. The endothelium was generally intact and resembled negative controls.

Corneas treated with the test substance, Z, for ten minutes showed diffuse sloughing of the full epithelium. There were necrotic epithelial remnants occasionally adjacent, or loosely attached, to the basal lamina. The stroma was slightly thicker than the positive controls. There was a moderate to marked expansion of the upper 50% of the stroma and mild focal expansion of the lower stroma. The upper stroma contained numerous necrotic keratocytes with pyknotic or vacuolated nuclei, and/or eosinophilic cytoplasm. There were scattered keratocytes in the lower stroma with vacuolated nuclei. The endothelium was generally intact, resembling the negative

control. However, there were a few foci with enlarged, hyperchromatic nuclei.

Corneas treated with the test substance, AA, for three minutes had an epithelium which resembled the positive controls, with hypereosinophilic squamous layer, vacuolated nuclei and cytoplasm in the wing and basal layers, and multifocal separation of the basal layers from the basal lamina. The epithelium was probably not viable at the time of fixation. The stroma was slightly thicker that the negative controls. In the upper 25% of the stroma there was minimal to mild expansion of the collagen matrix and scattered keratocytes with vacuolated nuclei and/or eosinophilic cytoplasm. Immediately subjacent to the epithelium were a small number of keratocytes with pyknotic nuclei. The endothelium was generally intact and resembled the negative controls.

Corneas treated with the test substance, AA, for 10 minutes had an epithelium which resembled the positive controls, with hypereosinophilic squamous layer, vacuolated nuclei and cytoplasm in the wing and basal layers, and multifocal separation of the basal layers from the basal lamina. The hypereosinophilic squamous layer was thicker and more prominent than in the three minute exposure group. The epithelium was probably not viable at the time of fixation. The stroma was slightly thicker than the positive controls. In the upper third of the stroma there was marked expansion of the collagen matrix and notable condensation of collagen. There was mild to moderate expansion of the middle third and mild focal expansion of the lower third of the stroma. In the upper stroma there were numerous keratocytes with vacuolated nuclei and occasional keratocytes with pyknotic nuclei. In the lower stroma, there were scattered keratocytes with eosinophilic cytoplasm. The endothelium was generally intact and resembled the negative control.

Histopathological evaluation of the corneas for this study was performed at Charles River Laboratories, Pathology Associates (PAI)-Maryland. The report detailing the findings can be found in Appendix C.

STUDY CONCLUSION

The following classification system was established by Sina et al.[1] based on studies with a wide range of test materials. While this classification system provides a good initial guide to interpretation of these *in vitro* data, these specific ranges may not be applicable to all classes of materials.

In Vitro Score:

from 0 to 25	= mild irritant
from 25.1 to 55	= moderate irritant
from 55.1 and above	= severe irritant

The *in vitro* scores for the test substance, Z, were 12.8 (3 minute exposure) and 31.6 (10 minute exposure), primarily resulting from increases in fluorescein permeability. Based on the classification established by Sina *et al* (1995), the test material would be classified as a moderate irritant. This classification is supported by the histopathological evaluation. The depth and degree of injury extended through the full thickness of the cornea. Histological evaluation showed diffuse sloughing of the full epithelium with necrotic epithelial remnants occasionally adjacent, or loosely attached, to the basal lamina. The stroma was slightly thicker than the positive control with moderate to marked expansion of the upper 50% of the stroma and mild focal expansion of the lower stroma. The upper stroma contained numerous necrotic keratocytes with pyknotic or vacuolated nuclei and/or eosinophilic cytoplasm. Additionally, there were scattered keratocytes in the lower stroma with vacuolated nuclei. The endothelium, while generally intact, there were a few foci with enlarged, hyperchromatic nuclei.

The *in vitro* scores for the test substance, AA, were 40.8 (3 minute exposure) and 75.0 (10 minute exposure), resulting from moderate increases in both opacity and fluorescein permeability. Based on the classification established by Sina *et a*l (1995), the test material would be classified as a severe irritant. This classification is supported by the histopathological evaluation. The depth and degree of injury extended through the full thickness of the cornea. Histological evaluation exhibited an epithelium which resembled the positive controls, with hypereosinophilic squamous layer, vacuolated nuclei and cytoplasm in the wing and basal layers, and multifocal separation of the basal layers from the basal lamina. The hypereosinophilic squamous layer was thicker and more prominent than the three minute exposure group and was probably not viable at the time of fixation. The stroma was slightly thicker than the positive controls. The upper third of the stroma showed a marked expansion of the collagen matrix and notable condensation of collagen. There was a mild to moderate expansion of the middle third and mild focal expansion of the lower third of the stroma. In the upper stroma there were numerous keratocytes with vacuolated nuclei and occasional keratocytes with pyknotic nuclei. In

[1]Sina, J.F., Galer, D.M., Sussman, R.G., Gautheron, P.D., Sargent, E.V., Leong, B., Shah, P.V., Curren, R.D., and Miller, K. (1995) A collaborative evaluation of seven alternatives to the Draize eye irritation test using pharmaceutical intermediates. **Fundamental and Applied Toxicology** 26:20-31.

the lower stroma there were scattered keratocytes with eosinophilic cytoplasm. The endothelium was generally intact and resembled the negative controls.

REPORT SUBMITTED BY:

_____ _____

Study Director

APPENDIX A

APPENDIX B

Performed on August 15, 2006 Study No. 06AG29-AG30.350025

BOVINE CORNEAL OPACITY AND PERMEABILITY ASSAY

OPACITY SCORE

TA #	CORNEA #	INITIAL	FINAL	CHANGE	CORRECTED	AVG	STDEV
06AG29	34	3	13	10	9.0		
Neat	36	4	5	1	0.0		
3 minutes	37	4	9	5	4.0	4.3	4.5
06AG29	39	3	10	7	6.0		
Neat	41	4	11	7	6.0		
10 minutes	43	4	14	10	9.0	7.0	1.7
06AG30	44	4	29	25	24.0		
Neat	46	3	29	26	25.0		
3 minutes	47	3	23	20	19.0	22.7	3.2
06AG30	48	4	49	45	44.0		
Neat	50	6	56	50	49.0		
10 minutes	52	5	47	42	41.0	44.7	4.0
Neg. Control	1	4	5	1	NA		
Sterile, DI water	2	4	5	1	NA	1.0	
60 minutes							
Pos. Control	7	4	31	27	26.0		
Ethanol	8	3	36	33	32.0		
10 minutes	10	5	37	32	31.0	29.7	3.2
	*4	5					
	*5	3					
	*11	3					
	*12	3					
	*13	4					
	*16	4					
	*17	3					
	*18	4					
	*19	6					
	*20	2					
	*21	5					
	*22	3					
	*24	4					
	*25	4					
	*26	6					
	*28	4					
	*29	4					
	*30	3					
	*31	4					
	*40	5					

Initial corneal opacity average: 4

* - Corneas not used in this assay, but used to find initial opacity average.
NA - Not Applicable

Performed on August 15, 2006 Study No. 06AG29-AG30.350025

PERMEABILITY SCORE

Neg. Control
Sterile, DI water
60 minutes

Cornea #	OD490
1	0.005
2	0.001
Avg.	0.003

Pos. Control
Ethanol
10 minutes

Cornea #	OD490	Dilution Factor	Corrected OD490
7	1.280	1	1.277
8	1.026	1	1.023
10	1.191	1	1.188
		Avg. =	1.163
		STDEV =	0.129

06AG29
Neat
3 minutes

Cornea #	OD490	Dilution Factor	Corrected OD490
34	1.038	1	1.035
36	0.466	1	0.463
37	0.200	1	0.197
		Avg. =	0.565
		STDEV =	0.428

06AG29
Neat
10 minutes

Cornea #	OD490	Dilution Factor	Corrected OD490
39	1.361	1	1.358
41	0.389	5	1.942
43	0.326	5	1.627
		Avg. =	1.642
		STDEV =	0.292

06AG30
Neat
3 minutes

Cornea #	OD490	Dilution Factor	Corrected OD490
44	1.155	1	1.152
46	1.323	1	1.320
47	1.148	1	1.145
		Avg. =	1.206
		STDEV =	0.099

06AG30
Neat
10 minutes

Cornea #	OD490	Dilution Factor	Corrected OD490
48	0.451	5	2.252
50	0.372	5	1.857
52	0.394	5	1.967
		Avg. =	2.025
		STDEV =	0.204

Performed on August 15, 2006 Study No. 06AG29-AG30.350025

IN VITRO SCORE

In Vitro Score = Mean Opacity Value + (15 x Mean OD490)

Test Article	Concentration	Exposure Period	Mean Opacity	Mean OD490	In vitro Score
06AG29	Neat	3 minutes	4.3	0.565	12.8
06AG29	Neat	10 minutes	7.0	1.642	31.6
06AG30	Neat	3 minutes	22.7	1.206	40.8
06AG30	Neat	10 minutes	44.7	2.025	75.0
Ethanol	Neat	10 minutes	29.7	1.163	47.1

APPENDIX C

APPENDIX D

CHARLES RIVER
LABORATORIES
Preclinical Services

FINAL HISTOPATHOLOGY REPORT

BOVINE CORNEAL OPACITY AND PERMEABILITY ASSAY WITH TWO TIME EXPOSURES AND HISTOLOGY

Testing Facility Study Number 06AG29-AG30.350025

HISTOLOGY SITE:

Charles River Laboratories,
Pathology Associates (PAI) - Maryland
15 Worman's Mill Court, Suite I
Frederick, MD 21701

TESTING FACILITY:

Institute for In Vitro Sciences, Inc.
21 Firstfield Road, Suite 220
Gaithersburg, MD 20878

SPONSOR:

February 23, 2007

CHARLES RIVER
LABORATORIES
Preclinical Services

Bovine Corneal Opacity and Permeability Assay with Two Time Exposures and Histology

Study Number: 06AG29-AG30.350025

QUALITY ASSURANCE STATEMENT

This histopathology project has been inspected and audited by the PAI Quality Assurance Unit (QAU) as required by the Good Laboratory Practice (GLP) regulations promulgated by the U.S. Environmental Protection Agency (EPA). The histopathology report is an accurate reflection of the recorded data. The following table is a record of the inspections/audits performed and reported by the QAU.

Date of Inspection	Phase Inspected	Date Findings Reported to Study Pathologist/ PAI Management	Date Findings Reported to Study Director/Study Director Management
09/05/06	Microtomy	09/12/06	09/12/06
11/29/06	Individual Animal Data and Supporting Documentation	11/29/06	11/29/06
11/29/06	Draft Histopathology Report	11/29/06	11/29/06
02/23/07	Final Histopathology Report	02/23/07	02/23/07

Hong Song

Hong Song
Quality Assurance Auditor

February 23, 2007
Date

1.0 Introduction

1.1 Overview

This report presents the pathology results of bovine corneas exposed to test and control substances for Institute for In Vitro Sciences, Inc (IIVS) Study Number 06AG29-AG30.350025. All test procedures and tissue harvests were performed at IIVS under the direction of Janet Luczak, M.G.A., Study Director. Histopathology was completed at Charles River Laboratories, Pathology Associates (PAI) - Maryland by Stewart B. Jacobson, DVM, DACVP.

1.2 Objective

The objective of this study was to evaluate the potential ocular irritancy/toxicity of the test article as measured by test article-induced changes in opacity, permeability to fluorescein, and tissue architecture in isolated bovine corneas. This report addresses the histopathology portion of the bovine corneal opacity and permeability (BCOP) assay.

2.0 Methods

2.1 Compliance Statement

The portion of this study performed by PAI was conducted under the U.S. EPA's FIFRA Good Laboratory Practice (GLP) regulations. Although digital photomicrographs are included as part of this report, the diagnoses and findings contained herein were obtained using the original histologic preparations, not the images. An electronic copy of this report (PDF format) is included. It is a representation of the pathology report hard copy; however, only the signed hard copy of the pathology report is considered raw data.

2.2 Archives

All pathology data and materials are maintained at Charles River Laboratories, Pathology Associates (PAI) – Maryland during the conduct of the histology portion of the study. Within three months of the issuance of the draft pathology report, or upon completion of the final pathology report, all data and materials generated by Charles River Laboratories, Pathology Associates (PAI) - Maryland, will have been shipped or delivered to the Institute for In Vitro Sciences, Inc. for archiving with the study.

2.3 Tissue Collection

According to the protocol, following treatment, corneas were placed in prelabeled cassettes in 10% neutral buffered formalin fixative. After 24 hours, the fixed tissues were transferred to PAI for slide preparation. Tissues were processed, bisected, embedded in paraffin and cut to 5 microns. Slides were stained with hematoxylin and eosin (H&E).

2.4 Basis for Histological Evaluation

Damage to the epithelial layer is generally the first change seen in the cornea since the test materials are applied topically to this "unprotected" epithelium. Each "layer" of the epithelium is scored for cell loss or damage. Figure 1 shows the structure of the epithelium from a control cornea. Changes to the surface epithelium (squamous and upper wing cell epithelium) in the BCOP assay are usually not predictive of lasting corneal changes *in vivo*. The loss of the squamous and upper wing cell layers in the excised bovine cornea by surfactant-based test articles appears to coincide with mild to moderate damage to the conjunctiva of the rabbit *in vivo*[1]. This type of damage is typically reversible in the rabbit. Many types of surfactants (e.g., sodium lauryl sulfate) are expected to lyse these cells so that they are progressively lost from the epithelial surface. The positive control, ethanol, provides an example of a different type of damage. In this case, the surface squamous epithelial cells are coagulated and fixed in place. The coagulated tissue stains heavily with eosin (see Figure 5). In addition, the wing and basal cells are vacuolated and the adhesion between the basal cells and the basal lamina is disrupted. Such changes can lead to sloughing of the epithelium. Lesions in the deep wing cell and basal cell layers, either by cell lysis or coagulation, are associated with more damage *in vivo*. Much of the available data come from studies on surfactants and show that loss of the bovine corneal epithelium is predictive of some corneal opacity in the rabbit.

Special effort has been made to detect changes in the stromal elements of the corneas. Jester[2], Maurer[3,4] and others have shown for a range of chemical classes that depth of injury in the early hours after exposure can be predictive of the eventual degree and duration of the ocular lesions. Epithelial damage alone, in the rabbit cornea, is associated with expected recovery provided the basal lamina is intact. Deep injury to the stroma has more serious consequences. Stromal damage may be manifest by several types of changes within the tissue. These may include swelling within the stromal collagen matrix and loss/damage to the keratocytes. These changes do not necessarily occur together. Stromal swelling may be detected by the presence of vacuole-like "holes" in the organized collagen matrix. Their appearance suggests that liquid has entered the matrix expanding space between the fibers. Examples of these vacuoles may be seen in Figure 7 where the positive control exposure has induced some stromal swelling. The depth and degree of vacuolization can be indicative of the degree of injury to the cornea and/or penetration of the test article into the tissue. Loss of the effective epithelial or endothelial barrier will allow water

---

[1] Gettings, SD, Lordo, RA, Hintze, KL, Bagley, DM, Casterton, PL, Chudkowski, M, Curren, RD, Demetrulias, JL, DiPasquale, LC, Earl, LK, Feder, PI, Galli, CL, Glaza, SM, Gordon, VC, Janus, J, Kurtz, PJ, Marenus, KD, Moral, J, Pape, WJW, Renskers, KJ, Rheins, LA, Roddy, MT, Rozen, MG, Tedeschi, JP, and Zyracki, J. (1996) The CFTA evaluation of alternatives program: an evaluation of *in vitro* alternatives to the Draize primary eye irritation test. (Phase III) Surfactant-based formulations. **Food and Chemical Toxicology** 34(1):79-117.

[2] Jester, JV, Li, HF, Petroll, WM, Parker, RD, Cavanaugh, HD, Carr, GJ, Smith, B, and Maurer, JK. (1998) Area and depth of surfactant-induced corneal injury correlates with cell death. **Investigative Ophthalmology & Visual Science** 39(6):922-936.

[3] Maurer, JK and Parker, RD. (1996) Light microscopic comparison of surfactant-induced eye irritation in rabbits and rats at three hours and recovery/day 35. **Toxicologic Pathology** 24(4):403-411.

[4] Maurer, JK, Parker, RD, and Jester, JV. (2002) Extent of initial corneal injury as the mechanistic basis for ocular irritation: key findings and recommendations for the development of alternative assays. **Regulatory Toxicology and Pharmacology** 36:106-117.

(medium) to enter the stroma and produce the collagen matrix vacuolization (swelling). Viable epithelium or endothelium will not only retard entry of the water but actively transport it out of the stroma. As the viability of epithelium declines, both the barrier and transport capacities are reduced. Thus, the amount of water accumulating in the upper stroma reflects the damage to the epithelium. When the full epithelial layer is lost over a section of stroma, stromal swelling can increase the stromal thickness by 50% or more over the control corneal stroma. Such changes are often observed when corneas treated with severe irritants are incubated for extended periods after exposure. Loss of the endothelium can also lead to appreciable deep stromal swelling. The loss may result from test article penetration or mechanical damage. Mechanical damage tends to be more focal (patchy) in nature while the true toxic response to the test article exposure tends to extend across much of the cornea. When focal (mechanical) damage is present, the majority of the collagen matrix vacuolization will be located in the deep stroma (just above Descemet's Membrane). In contrast, test article-induced toxicity to the endothelium should include both epithelial as well as endothelial damage so that collagen matrix vacuolization will be observed throughout the stroma. Such extreme damage may increase the stromal thickness two fold over the control corneal stroma thickness.

In vivo, the inflammatory response that follows deep initial injury can, itself, lead to permanent lesions through "scar" collagen deposition or neovascularization in the corneal stroma. The authors cited above have suggested that the damaged keratocytes are involved in initiating this inflammatory process. Classic inflammatory changes (e.g., inflammatory cell infiltration) are possible only in the presence of active circulation through the limbus. Since the isolated cornea has no source of inflammatory cells, the potential for test article-induced inflammation is judged by the changes in the extracellular matrix and particularly the keratocytes. Some forms of damage are more easily recognized than are others. Necrotic cell death, as might follow exposure to a strong alkaline, would be quite apparent since the cellular components rapidly break down. More subtle damage could also lead to a delayed cell death and release of inflammatory mediators. Nuclear changes (vacuolization [swelling], punctate chromatin condensation, pyknosis or karyorrhexis) are signs of this process. Cytoplasmic changes can also be informative. Vacuole formation and/or loss of basic elements (mRNA for example) are also indicative of the beginning of the degenerative process. The cell cytoplasm normally stains with both basophilic (hematoxylin) and acidophilic (eosin) stains. When the basic elements are lost, eosinophilic staining predominates. This type of change is reported as cytoplasmic eosinophilia. Harbell and Curren[5] have reported that mechanical removal of the corneal epithelium leads to marked stromal swelling (marked collagen matrix vacuolization). The keratocytes in the zone of marked swelling undergo changes as well. The nuclei become enlarged and the cytoplasmic staining is very eosinophilic. Thus, when test article-treated corneas show only this type of keratocyte change (with stromal swelling), the change may be the result of the stromal swelling rather than direct action of the test article on the cells.

Since depth of injury (both extracellular and cellular) to the stroma is important in the analysis, a means to describe the depth of injury is necessary as part of this analysis. Determining this depth is not always a straightforward process. Because of the topical application of the test article to the epithelium, one would expect that exposure to the stroma would progress from the area just

[5] Harbell, J and Curren, R (2005) Using histological evaluation to enhance the Bovine Corneal Opacity and Permeability (BCOP) assay. ALTEX 22 (Special Issue):236.

under Bowman's Layer down through the stroma to Descemet's Membrane. There is no external inflammatory process *in vitro*, so one might also expect the progression of damage to follow the progression of exposure. That means that damage to the stroma should first appear close to Bowman's Layer. As the damage to the stroma increases, deeper layers might be involved. This implies that one would want to express damage as progressing from the anterior (Bowman's Layer) to the posterior (Descemet's Membrane) and express it as a fraction of the total stromal depth involved. However, collagen matrix vacuolization can (and often does) increase total stromal thickness. Thus, measurements of the depth of a stromal lesion can be complicated by the change in overall stromal thickness. To account for stromal swelling, this depth may be estimated from the percentage of the stromal cross section that remained undamaged (starting at the posterior border). For this report, depth of stromal damage is reported simply in terms of relative depth (e.g., upper, middle and deep stroma).

Photomicrographs of the epithelium and stroma are intended to illustrate the degree of damage at the indicated depth. Where little or no damage to the stroma was detected, the area directly under Bowman's Layer was photographed since that is where one would expect to see damage if it had occurred. Images were captured using a Micropublisher 5.0 Digital Camera connected to an Olympus BX45 microscope and processed using Image-Pro Plus software version 5.1.2.

With the digital camera and associated software, it is possible to measure distances within the captured image. This feature can be used to measure corneal stromal thickness directly. Such measurements require a true cross-section of the cornea (i.e., a perpendicular section relative to the corneal surface plane). In some cases, a true cross-section is not available. The Descemet's Membrane thickness is used to estimate how close the section is to a true cross section. The appearance of an unusually thick membrane suggests that the section was cut at a tangent to the true cross section (or from a more mature animal). If the measured corneal section did not appear to be a true cross section, the observation was noted. Even in a true cross section, the isolated cornea is not of uniform thickness but rather shows peaks and valleys. Therefore, an effort has been made to select "representative" cross-sections that are neither extreme "peaks" nor "valleys" for measurement of corneal thickness. The values obtained should be considered "representative" of the treatment group rather than strict quantitative measures. Many more measurements would be required to provide a quantitative comparison. In some cases, the treated corneas will have lost most of their epithelium or it has been compromised (i.e., ethanol treatment). In those cases, the stromal thickness of the treated corneas should be compared to the stromal thickness of the negative control-treated corneas.

2.5 Histopathologic Results

The negative control corneas were treated for 60 minutes with sterile, deionized water (slides C0946-C0947). The negative control-treated epithelium was composed of three layers. The basal cell layer was a well-formed, columnar-cell region directly attached to the Bowman's Layer. The basal cells were always tightly attached to each other. Several layers of wing cells covered the columnar basal layer. In both of these layers, the cell nuclei showed diffuse chromatin without clear nucleoli. Asymmetric clear spaces ("nuclear halos") were occasionally observed around nuclei in the wing and basal cell layers. These were an artifact of tissue processing and unrelated to treatment. Rare mitotic figures were seen in the basal layer. The squamous layer was flattened

with limited cytoplasm and highly condensed nuclei (Figure 2).

The stromal elements in the negative control corneas showed well-organized collagen matrix fibers with dispersed keratocytes. Keratocyte nuclei showed a range of morphologies from moderate sized (smaller than the epithelial nuclei) with diffuse basophilic staining, to narrow, elongated and condensed with dark basophilic staining. Cytoplasmic staining, when it was visible, was moderately basophilic. Rarely cells with eosinophilic cytoplasmic staining were observed. Collagen bundles were generally parallel and well-ordered (Stroma just under Bowman's Layer, Figure 3).

The Descemet's Membrane was prominent. The endothelial layer could be seen in most sections and was reasonably well-maintained.

The cross section of a negative control-treated cornea, showing the general thickness of the whole cornea and stroma, is shown in Figure 4.

The positive control corneas (slides C0950-C0952), treated for 10 minutes with 100% ethanol, showed the classic pattern of squamous layer coagulation (darkening) and marked vacuolization in the wing and basal cell nuclei (Figure 5). The loss of adhesion between the basal cells (cell to cell) and the basal lamina was marked. The epithelium was probably not viable at the time of fixation. Overall, the positive control-treated corneas were thicker than the negative control-treated corneas (Figure 6). In the stroma directly below Bowman's Layer, the collagen matrix showed slight hypereosinophilic staining suggestive of some coagulation. Below this zone, moderate/marked collagen matrix vacuolization extended past 50% depth. In the upper stroma, there was a decrease in the density of viable keratocytes (Figure 7) as reflected by a marked increase in the frequency of keratocytes showing nuclear vacuolization (Figure 8). In the stroma below mid depth, the keratocytes showed a moderate increase in the frequency of cells with enlarged nuclei and cytoplasmic eosinophilia (Figure 9). The endothelial cells were generally intact (similar to the negative control-treated corneas).

Table 1 presents the results for the test article evaluated.

Table 1
Histological Observations of the Test Article-Treated Corneas

IIVS Number	Sponsor's Designation	Exposure Time (minutes)/ Post-Exposure Incubation (minutes)	Observations	Figure #
06AG29 Slides C0968-C0970	Z	3/120	**Epithelium**: There was diffuse loss of the squamous layer and blanching of exposed superficial wing cells (Figure 10). Multifocally, the	10-12

IIVS Number	Sponsor's Designation	Exposure Time (minutes)/ Post-Exposure Incubation (minutes)	Observations	Figure #
			cytoplasm of deeper wing and basal cells were finely vacuolated. **Stroma**: The stroma was similar thickness to negative controls (Figure 11). Immediately subjacent to the epithelium there was minimal expansion of the collagen matrix and scattered keratocytes with eosinophilic cytoplasm (Figure 12). The remainder of the stroma resembled negative controls. **Endothelium**: The endothelium was generally intact and resembled negative controls.	
06AG29 Slides C0971-C0973	Z	10/120	**Epithelium**: There was diffuse sloughing of the full epithelium (Figure 13). There were necrotic epithelial remnants occasionally adjacent, or loosely attached, to the basal lamina. **Stroma**: The stroma was slightly thicker than positive controls (Figure 14). There was moderate to marked expansion of the upper 50% of the stroma (Figure 15) and mild focal expansion of the lower stroma. The upper stroma contained numerous necrotic keratocytes with pyknotic or vacuolated nuclei, and/or eosinophilic cytoplasm (Figure 15). There were scattered keratocytes in the lower stroma with vacuolated nuclei. **Endothelium**: The endothelium was generally intact. While the endothelium resembled negative	13-15

IIVS Number	Sponsor's Designation	Exposure Time (minutes)/ Post-Exposure Incubation (minutes)	Observations	Figure #
			controls, there were a few foci with enlarged, hyperchromatic nuclei.	
06AG30 Slides C0974-C0976	AA	3/120	**Epithelium**: The epithelium resembled positive controls, with hypereosinophilic squamous layer, vacuolated nuclei and cytoplasm in the wing and basal layers, and multifocal separation of the basal layers from the basal lamina (Figure 16). The epithelium was probably not viable at the time of fixation. **Stroma**: The stroma was slightly thicker than negative controls (Figure 17). In the upper 25% of the stroma there was minimal to mild expansion of the collagen matrix and scattered keratocytes with vacuolated nuclei and/or eosinophilic cytoplasm (Figure 18). Immediately subjacent to the epithelium were a small number of keratocytes with pyknotic nuclei (Figure 19). The remainder of the stroma resembled negative controls. **Endothelium**: The endothelium was generally intact and resembled negative controls.	16-19
06AG30 Slides C0977-C0979	AA	10/120	**Epithelium**: The epithelium resembled positive controls, with hypereosinophilic squamous layer, vacuolated nuclei and cytoplasm in the wing and basal layers, and multifocal separation of the basal layers from the basal lamina (Figure 20). The hypereosinophilic squamous layer was thicker and more	20-24

IIVS Number	Sponsor's Designation	Exposure Time (minutes)/ Post-Exposure Incubation (minutes)	Observations	Figure #
			prominent than the 06AG30 three minute exposure group. The epithelium was probably not viable at the time of fixation. **Stroma**: The stroma was slightly thicker than the positive controls (Figure 21). In the upper third of the stroma there was marked expansion of the collagen matrix and notable condensation of collagen (Figure 22). There was mild to moderate expansion of the middle third and mild focal expansion of the lower third of the stroma. In the upper stroma there were numerous keratocytes with vacuolated nuclei (Figure 22) and occasional keratocytes with pyknotic nuclei (Figure 23). In the lower stroma there were scattered keratocytes with eosinophilic cytoplasm (Figure 24). **Endothelium**: The endothelium was generally intact and resembled negative controls.	

The figures displayed on the subsequent pages of this pathology report are representative H&E-stained cross-sections presented at the magnifications indicated in each image caption. A 100 um bar appears on each micrograph for reference. Arrows included in some of the figures identify examples of the lesions noted, however not all lesions in all micrographs are marked. Vertical double-headed arrows mark the limits of the epithelium in the appropriate figures.

Stewart B. Jacobson, DVM DACVP
Principal Investigator

2/23/07
Date

Figure 1. An example of a control cornea showing the three layers of the epithelium, Bowman's Layer and the upper stroma (H&E)

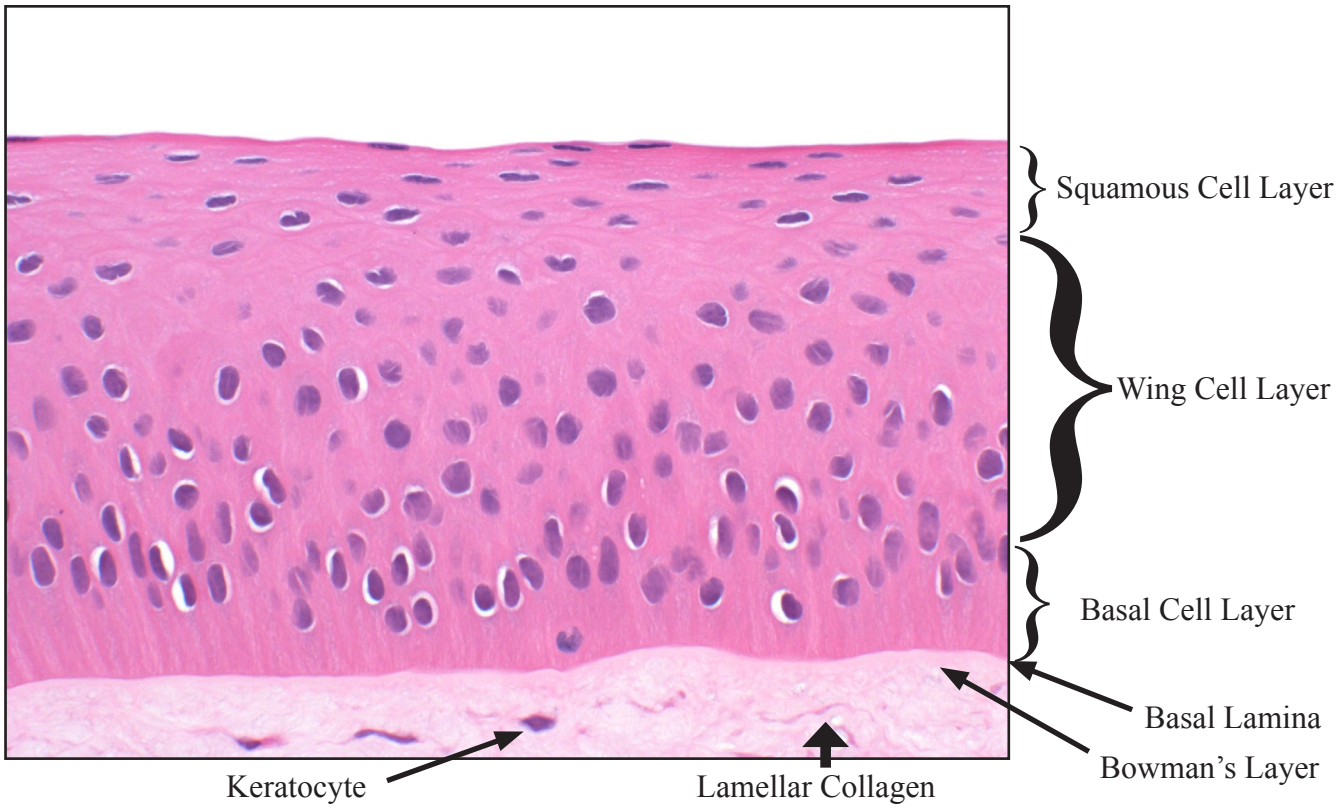

Figure 2. Negative Control (sterile, deionized water, 60 minute exposure/60 minute post incubation) - Epithelium (Slide C0946-1.1RC, 40x, H&E)

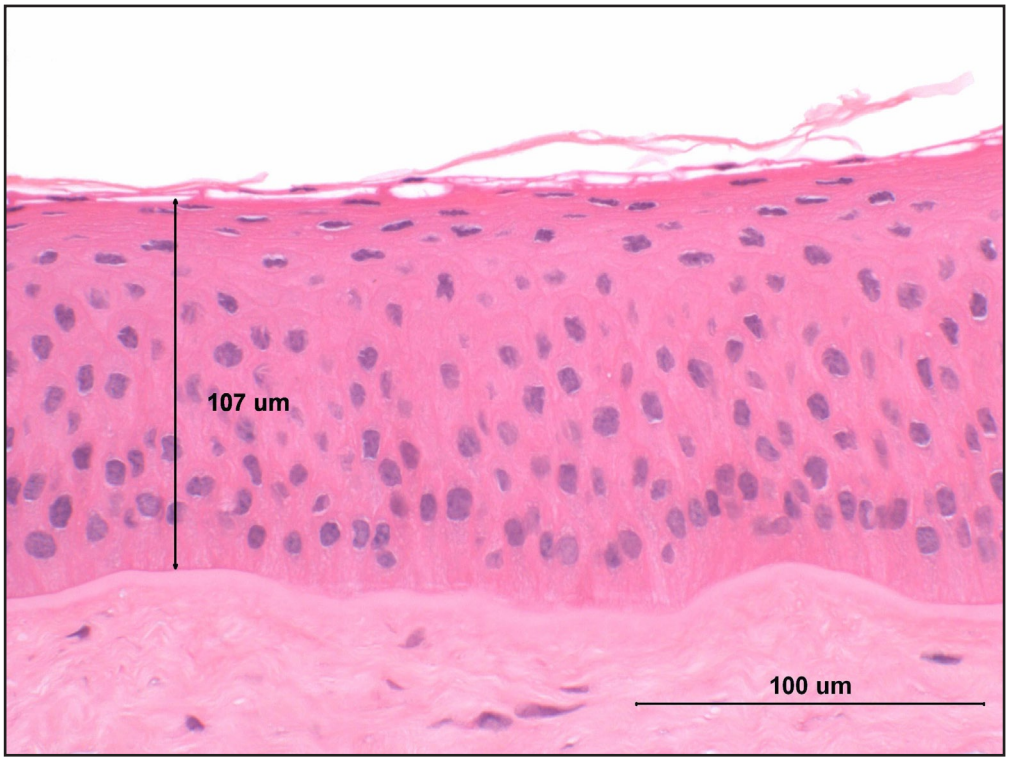

Figure 3. Negative Control (sterile, deionized water, 60 minute exposure/60 minute post incubation) - Stroma directly below Bowman's Layer (Slide C0946-1.1RC, 40x, H&E)

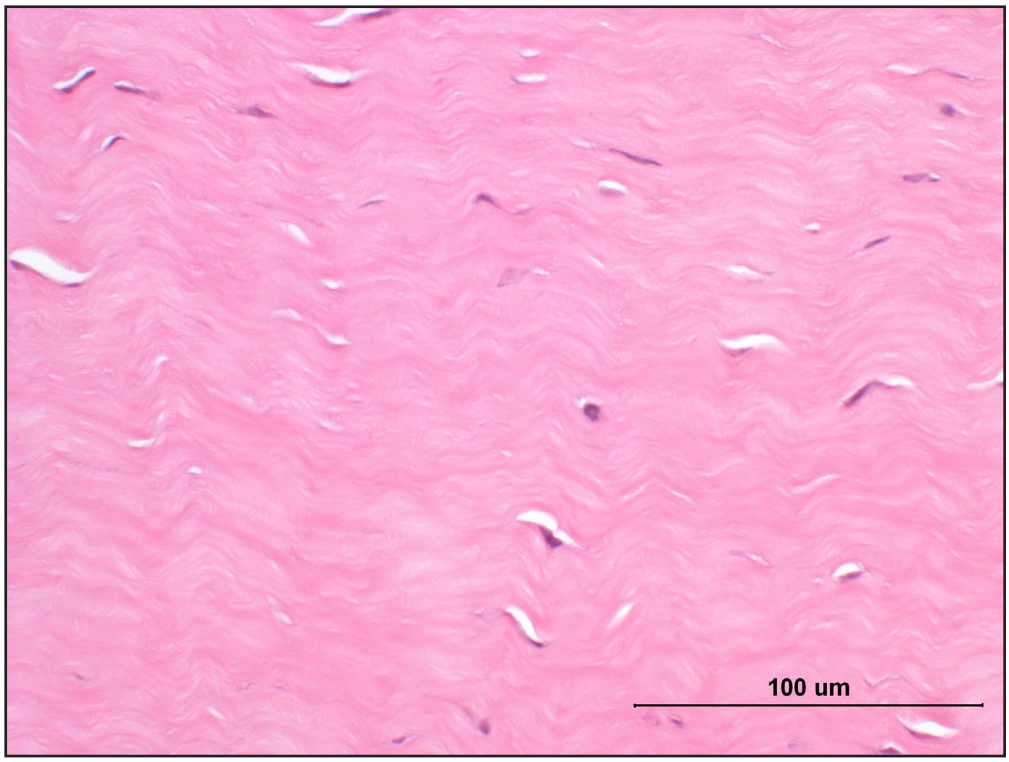

Figure 4. Negative Control (sterile, deionized water, 60 minute exposure/60 minute post incubation) - Full thickness (Slide C0946-1.1RC, 4x, H&E)

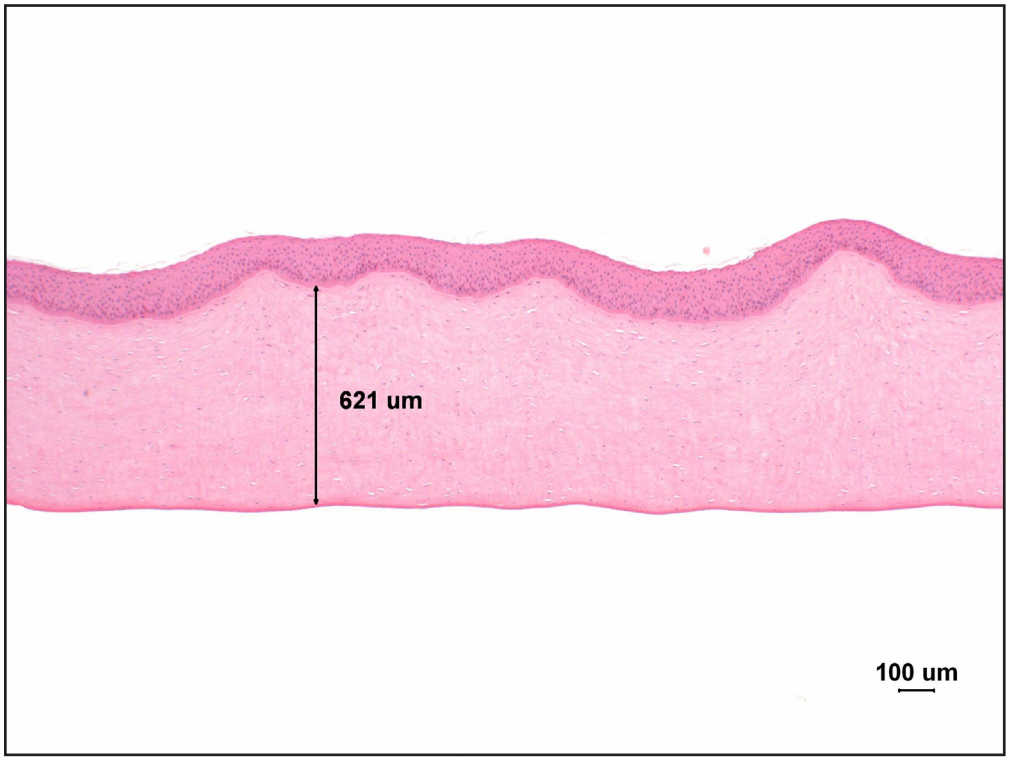

Figure 5. Positive Control (100% ethanol, 10 minute exposure/120 minute post incubation) - Epithelium with marked coagulation of the squamous layer (A ⟶) and vacuolation of the wing and basal cell nuclei (B ⟶) (Slide C0950-1, 40x, H&E)

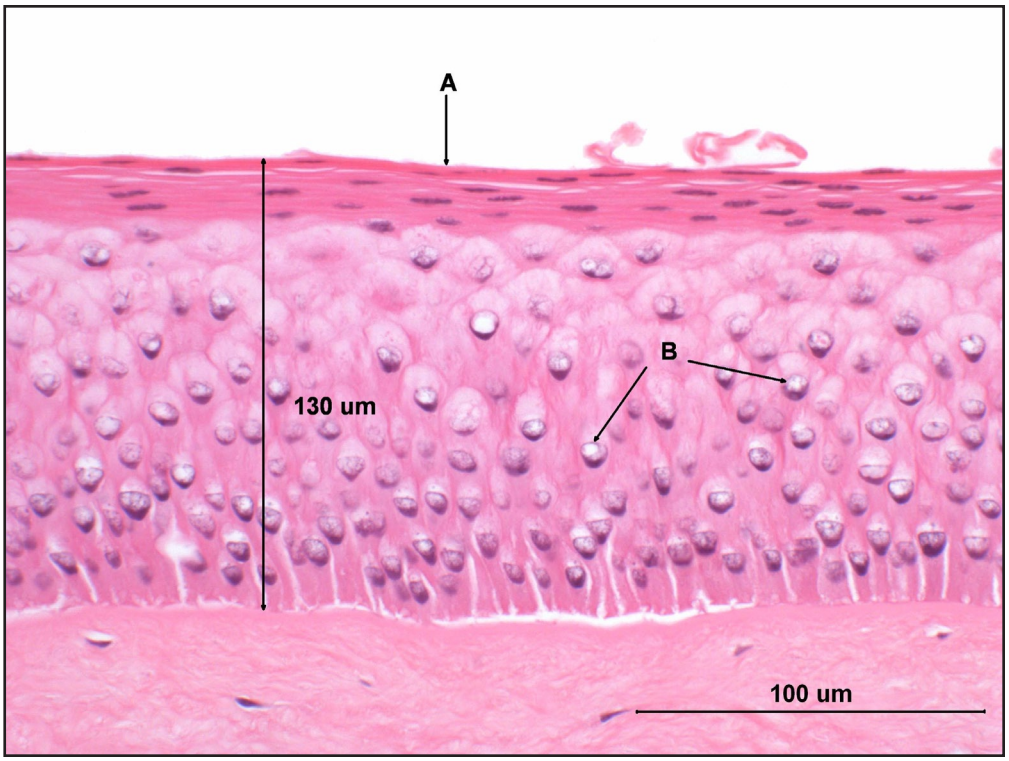

Figure 6. Positive Control (100% ethanol, 10 minute exposure/120 minute post incubation) - Full thickness (Slide C0951-1, 4x, H&E)

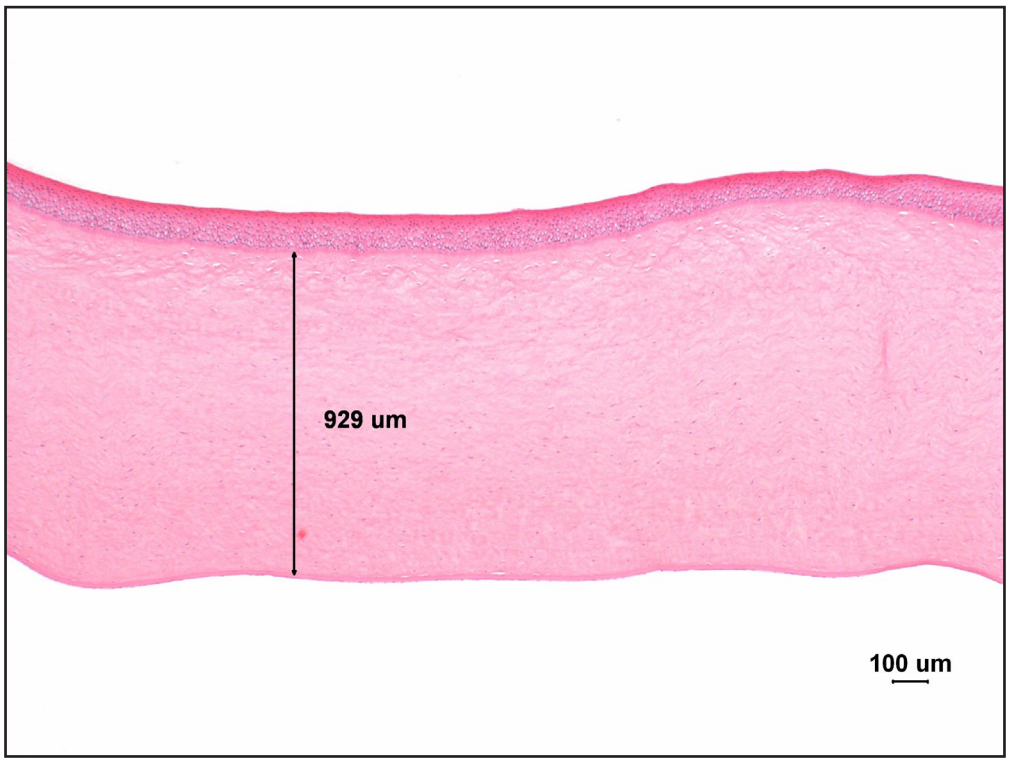

Figure 7. Positive Control (100% ethanol, 10 minute exposure/120 minute post incubation) - Upper stroma with expansion of the collagen matrix (⟶) (Slide C0951-1, 40x, H&E)

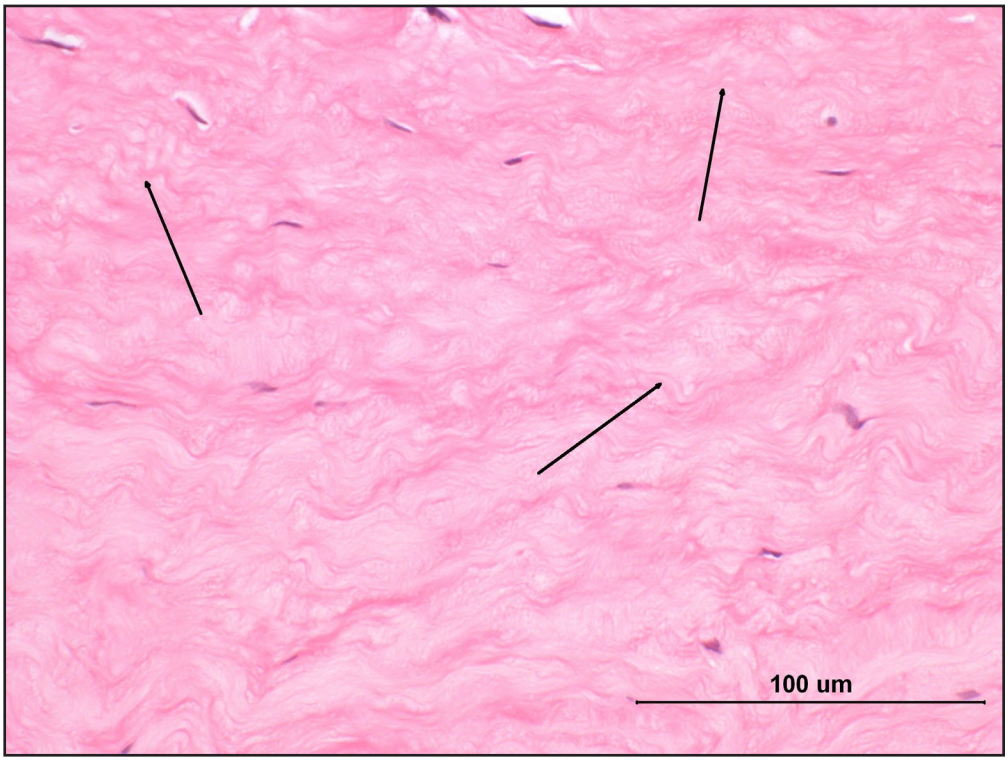

Figure 8. Positive Control (100% ethanol, 10 minute exposure/120 minute post incubation) - Superficial stroma with vacuolated keratocyte nuclei (⟶) (Slide C0951-1, 40x, H&E)

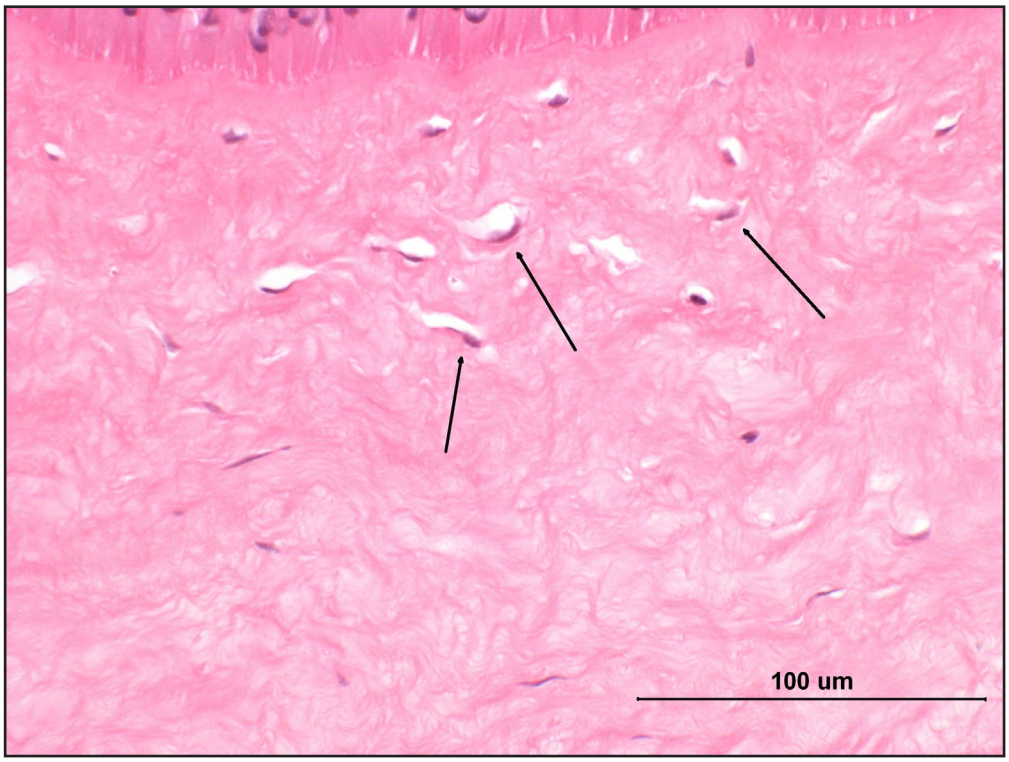

Figure 9. Positive Control (100% ethanol, 10 minute exposure/120 minute post incubation) - Mid stroma with eosinophilic cytoplasm (⟶) (Slide C0951-1, 40x, H&E)

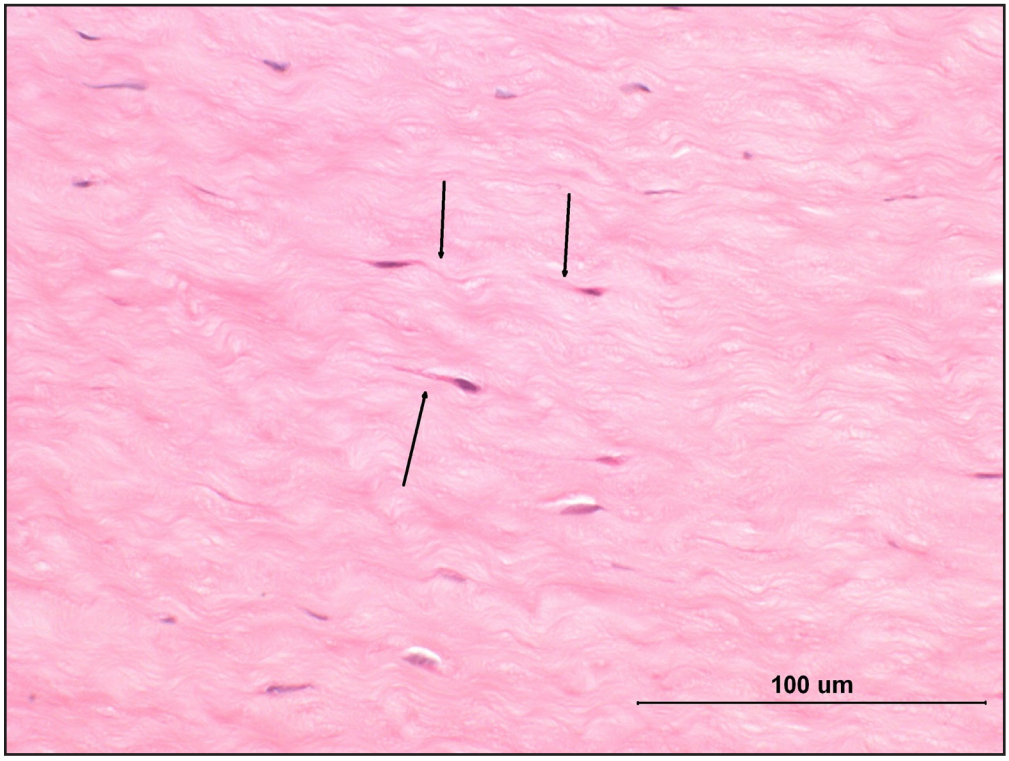

Figure 10. 06AG29, Z (3 minute exposure/120 minute post incubation) - Epithelium with loss of the squamous layer (Slide C0970-1.1RC, 40x, H&E)

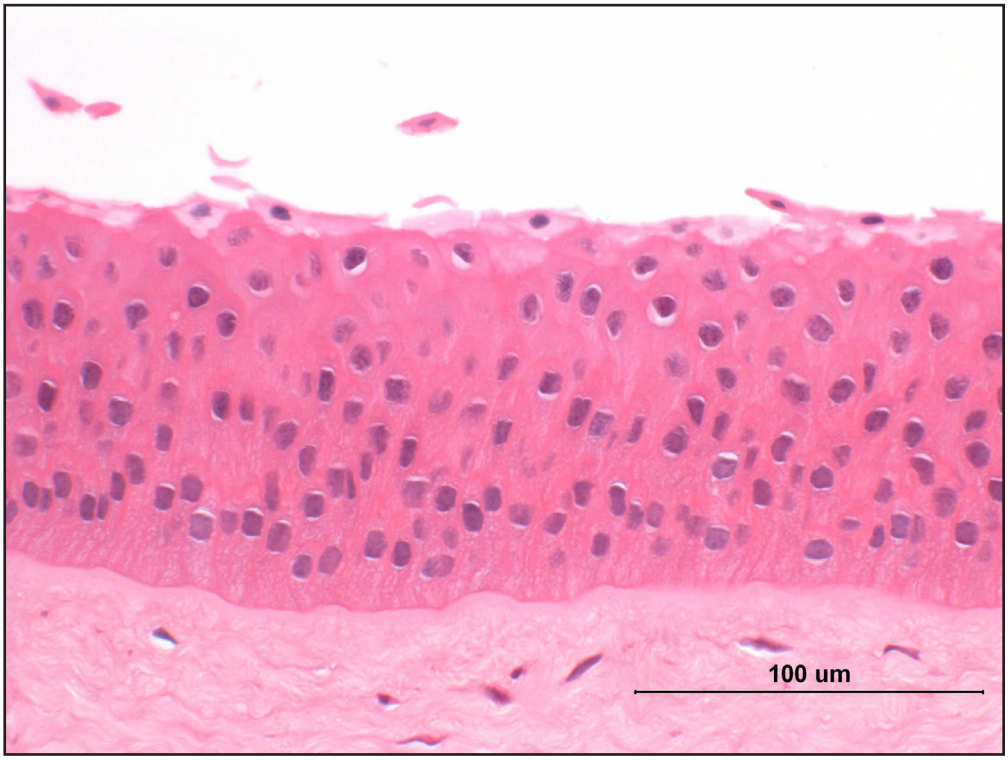

Figure 11. 06AG29, Z (3 minute exposure/120 minute post incubation) - Full thickness (Slide C0970-1.1RC, 4x, H&E)

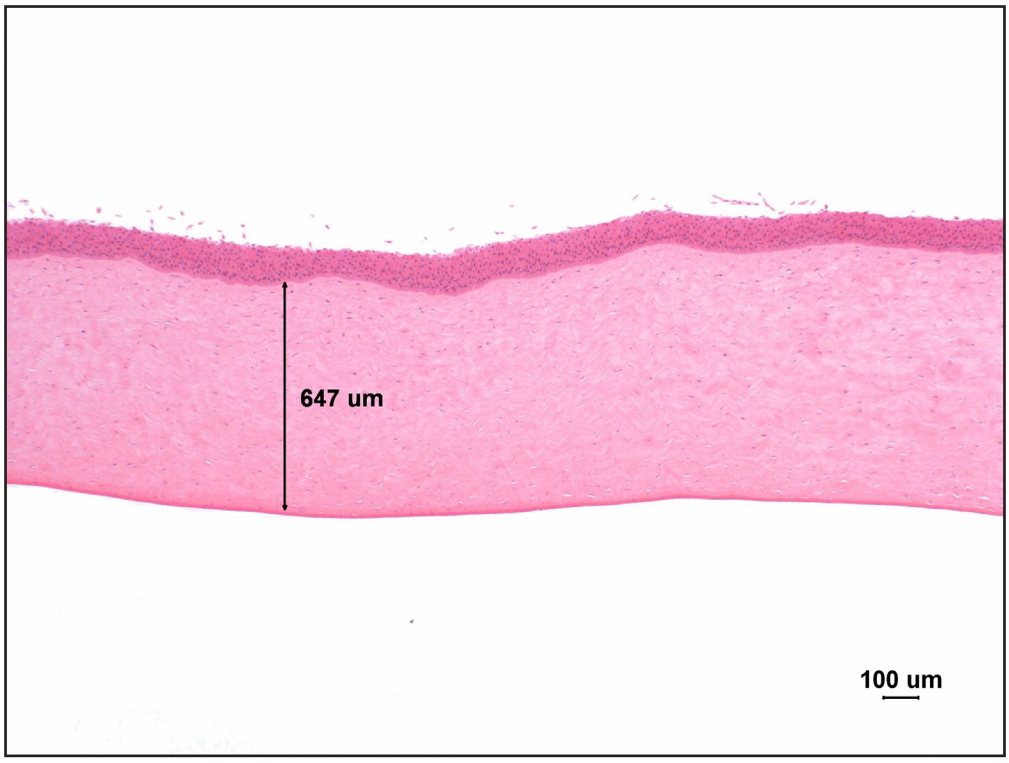

Figure 12. 06AG29, Z (3 minute exposure/120 minute post incubation) - Superficial stroma with expansion of the collagen matrix (A ⟶) and keratocytes with eosinophilic cytoplasm (B ⟶) (Slide C0970-1.1RC, 40x, H&E)

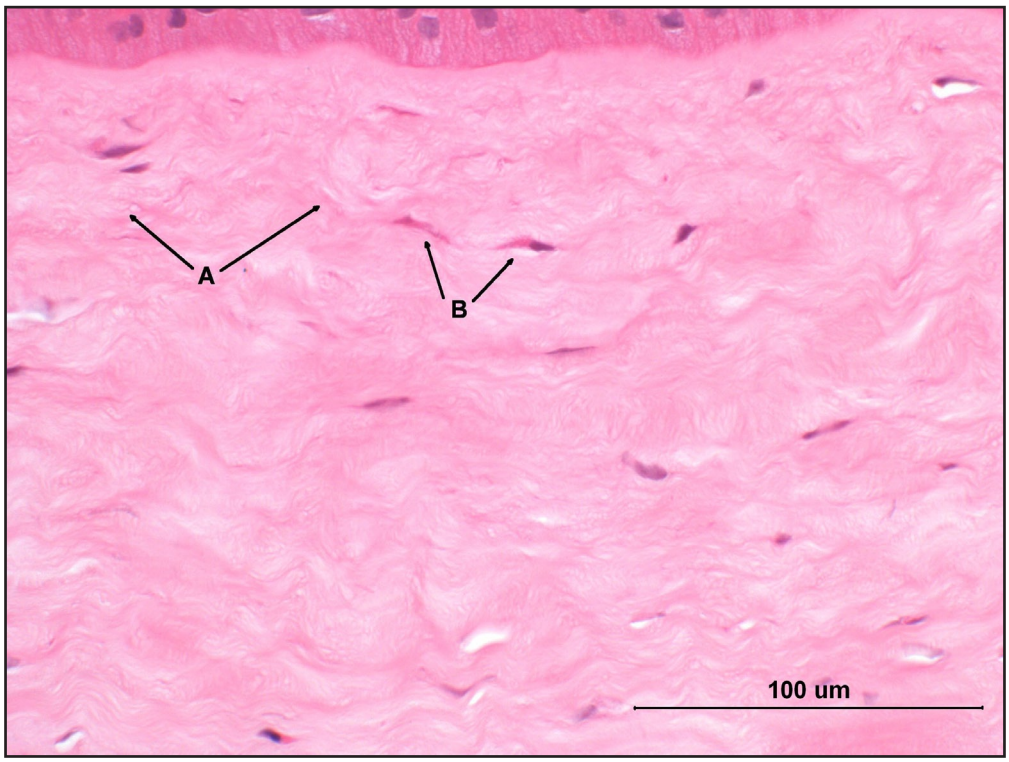

Figure 13. 06AG29, Z (10 minute exposure/120 minute post incubation) - Epithelium with full thickness necrosis and sloughing (Slide C0973-1.1RC, 40x, H&E)

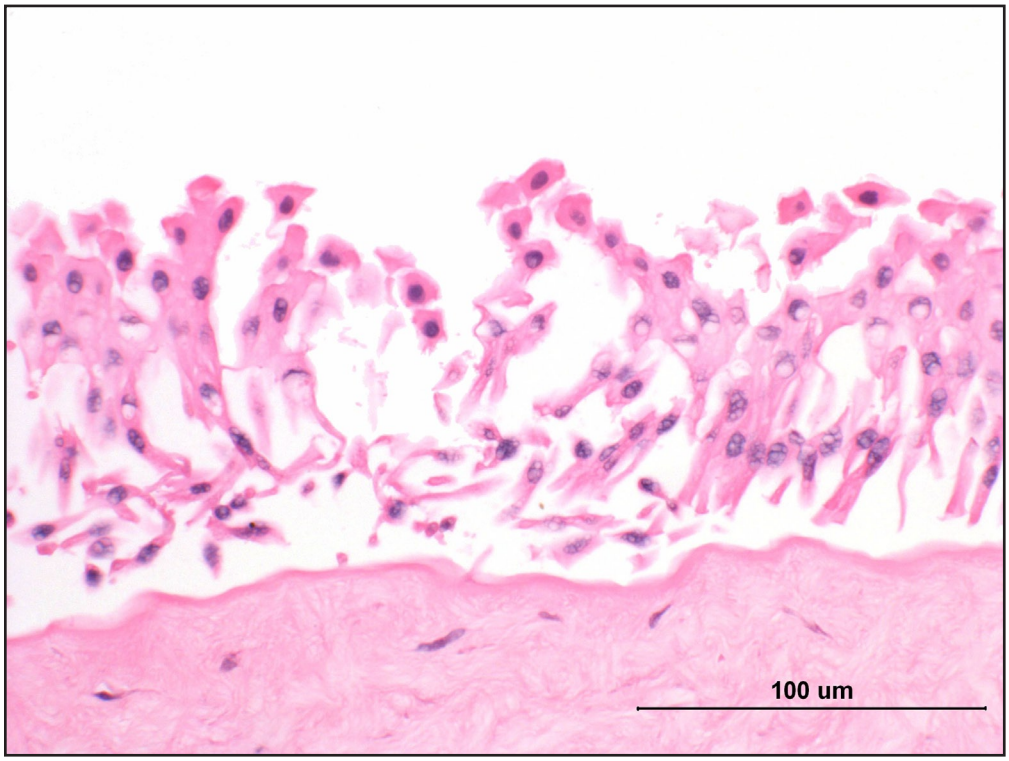

Figure 14. 06AG29, Z (10 minute exposure/120 minute post incubation) - Full thickness (Slide C0973-1.1RC, 4x, H&E)

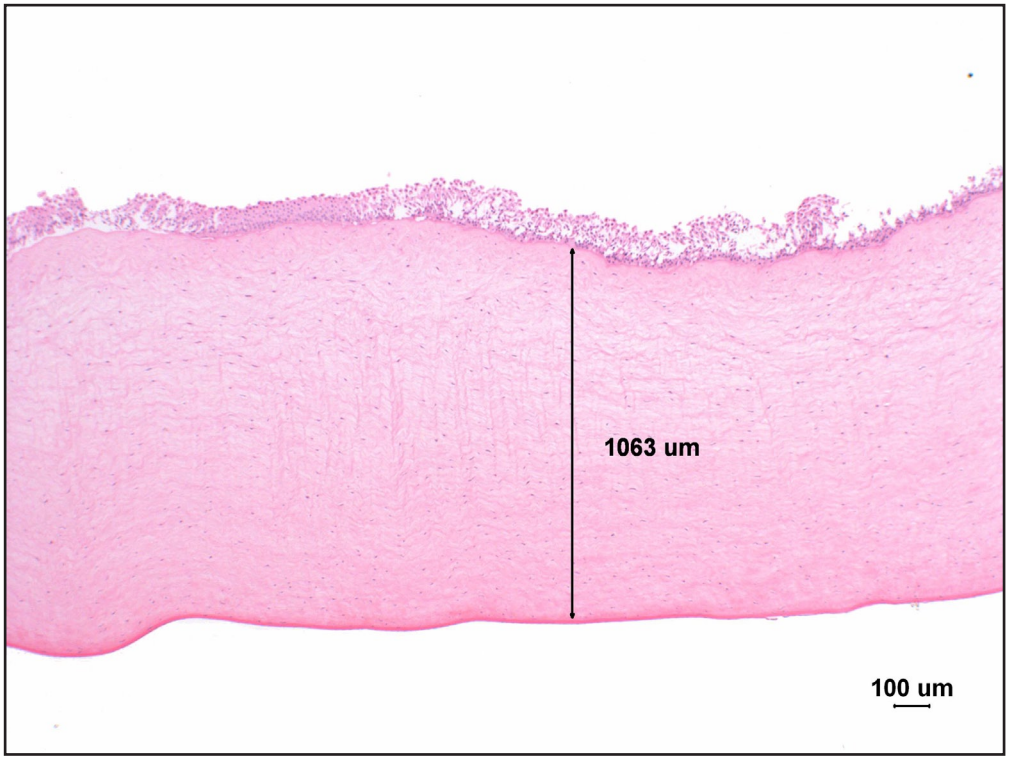

Figure 15. 06AG29, Z (10 minute exposure/120 minute post incubation) - Upper stroma with expansion of the collagen matrix (A ⟶), nuclear pyknosis (B ⟶), vacuolated keratocyte nuclei (C ⟶), and eosinophilic cytoplasm (D ⟶) (Slide C0973-1.1RC, 40x, H&E)

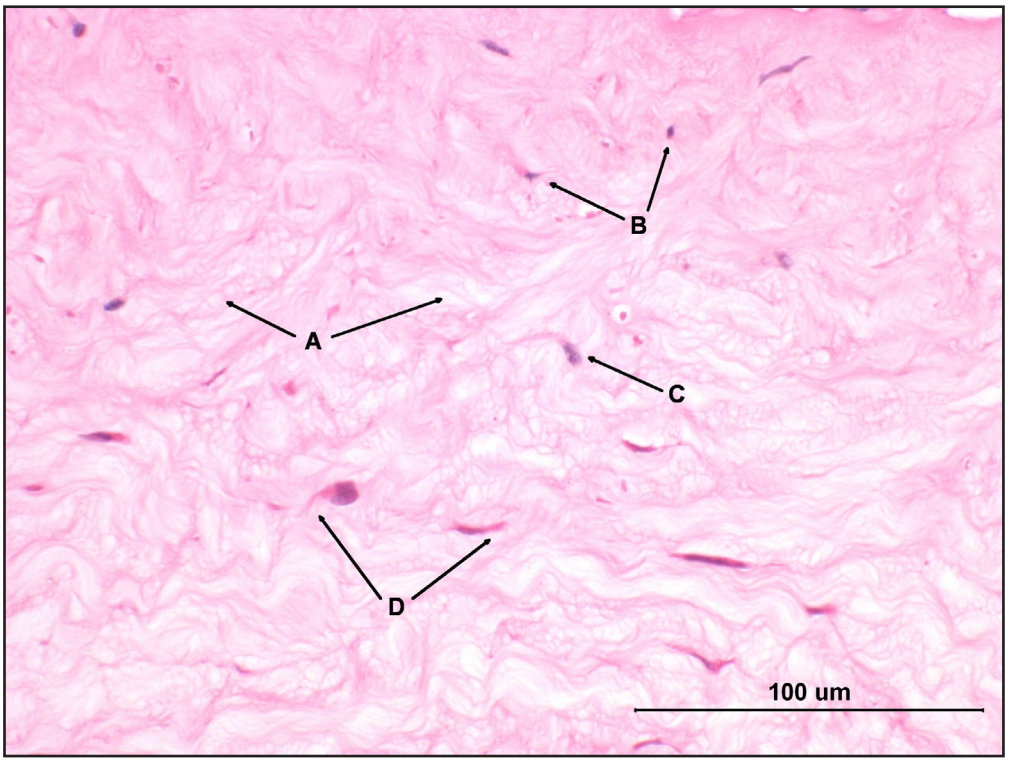

Figure 16. 06AG30, AA (3 minute exposure/120 minute post incubation) - Epithelium with hyper-eosinophilic squamous layer (A ⟶) and separation of the basal layer (B ⟶) (Slide C0975-1.1RC, 40x, H&E)

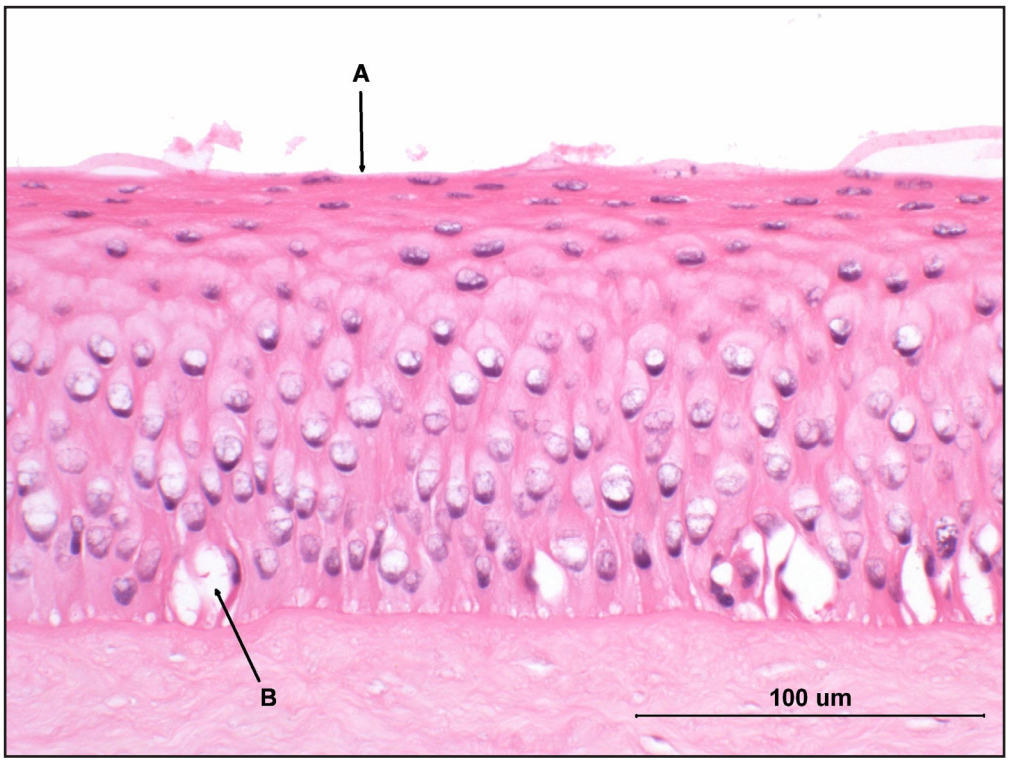

Figure 17. 06AG30, AA (3 minute exposure/120 minute post incubation) - Full thickness (Slide C0975-1.1RC, 4x, H&E)

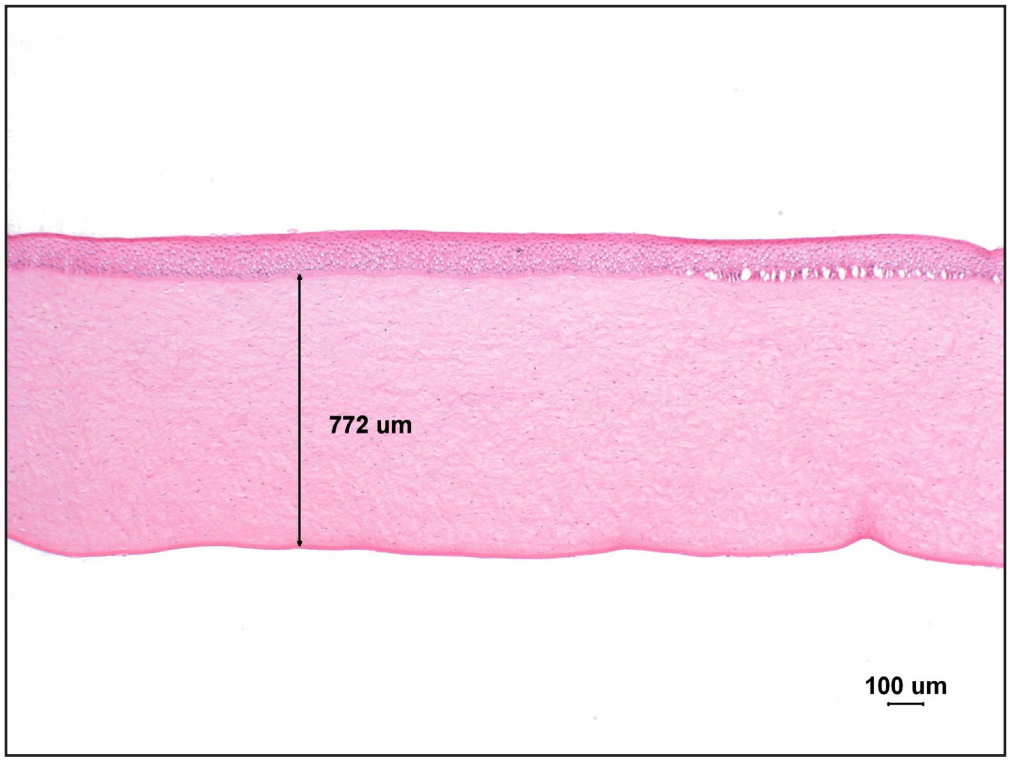

Figure 18. 06AG30, AA (3 minute exposure/120 minute post incubation) - Upper stroma with expansion of the collagen matrix (A ⟶), vacuolation of keratocyte nuclei (B ⟶), and eosinophilic keratocyte cytoplasm (C ⟶) (Slide C0975-1.1RC, 40x, H&E)

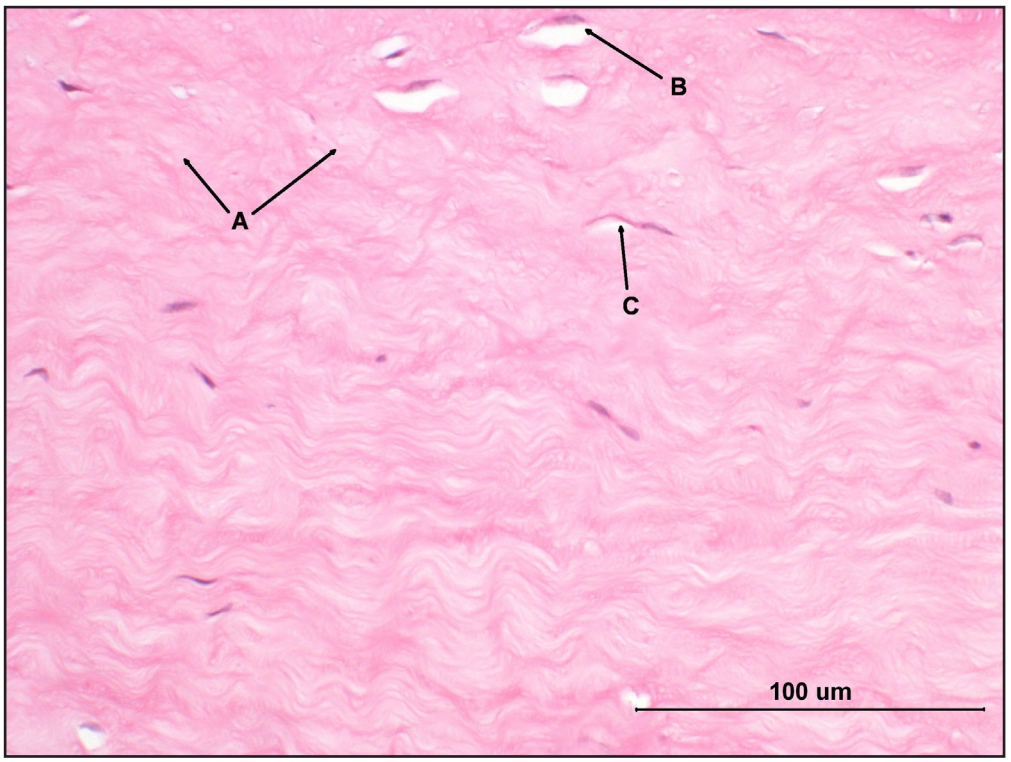

Figure 19. 06AG30, AA (3 minute exposure/120 minute post incubation) - Upper stroma with pyknotic keratocyte nuclei (⟶) (Slide C0975-1.1RC, 40x, H&E)

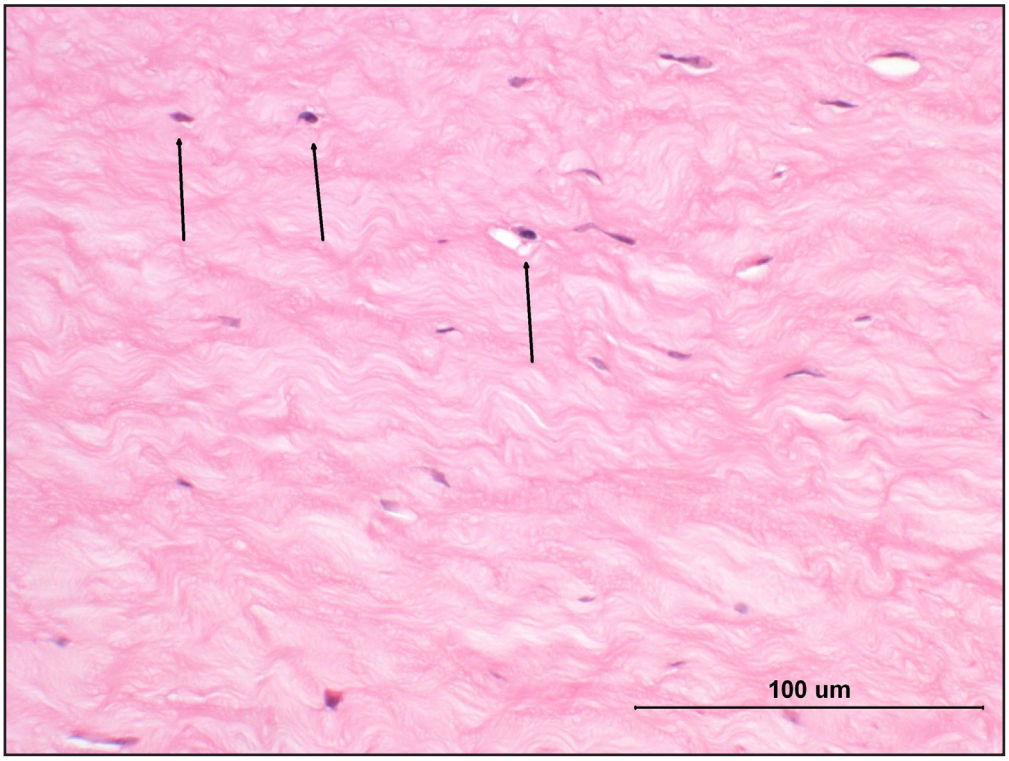

Figure 20. 06AG30, AA (10 minute exposure/120 minute post incubation) - Epithelium with hyper-eosinophilic squamous layer (A ⟶) and separation of the basal layer (B ⟶) (Slide C0977-1, 40x, H&E)

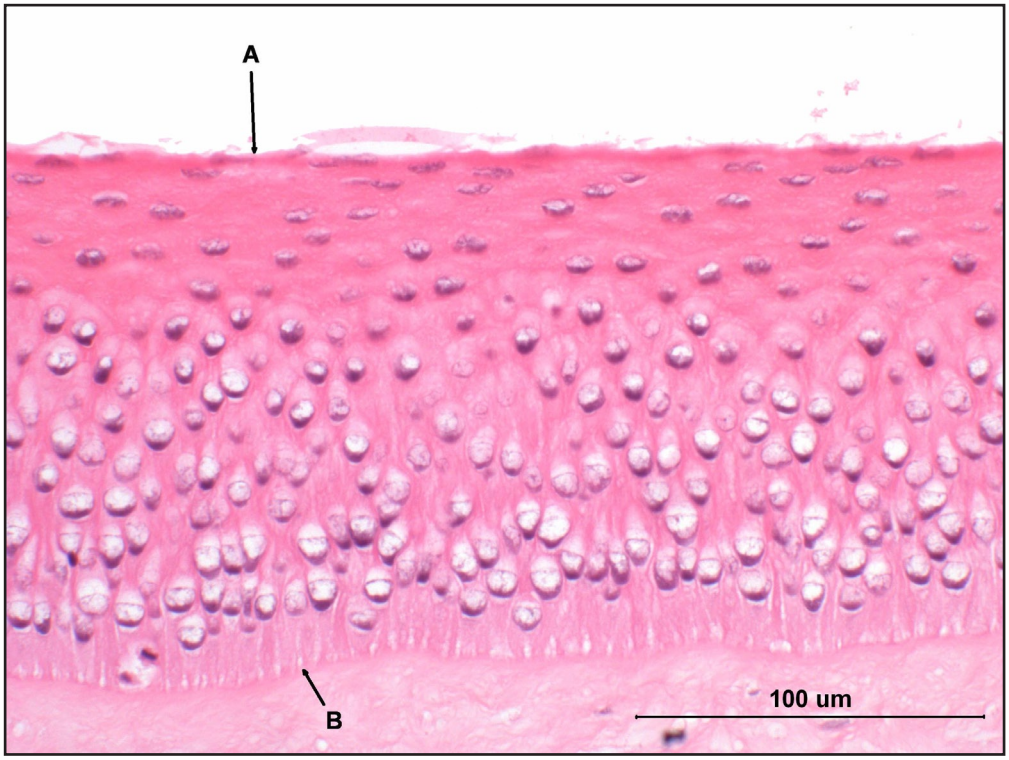

Figure 21. 06AG30, AA (10 minute exposure/120 minute post incubation) - Full thickness (Slide C0979-1, 4x, H&E)

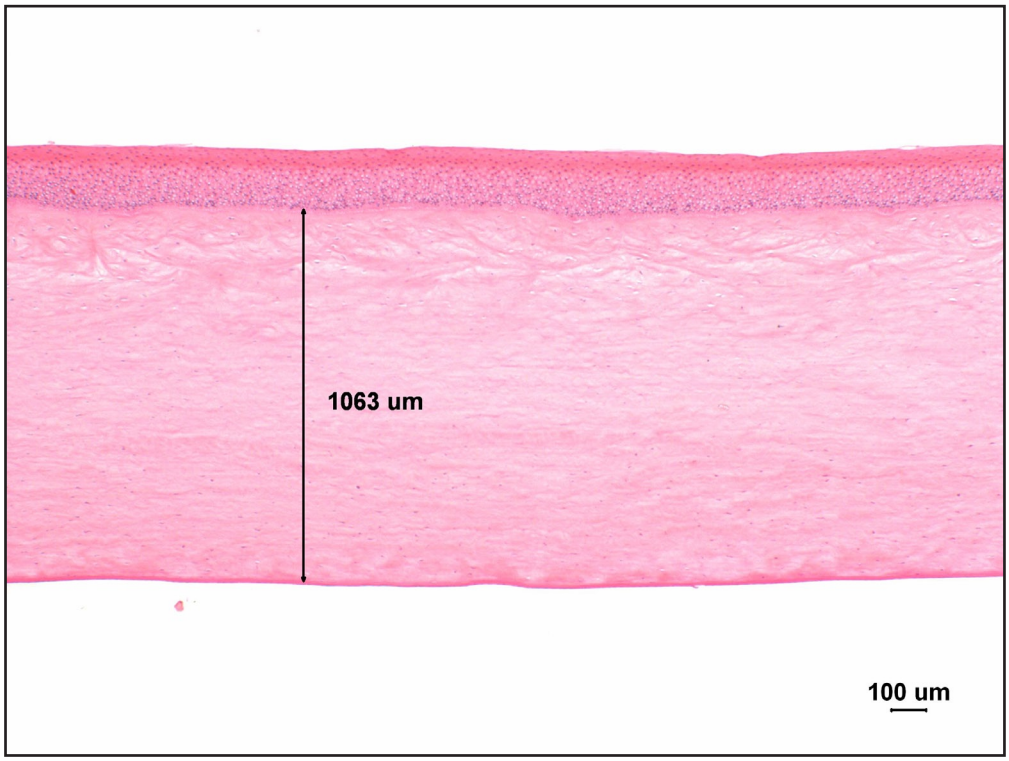

Figure 22. 06AG30, AA (10 minute exposure/120 minute post incubation) - Upper stroma with expansion of the collagen matrix (A ⟶), condensation of collagen (B ⟶), and vacuolation of keratocyte nuclei (C ⟶) (Slide C0979-1, 40x, H&E)

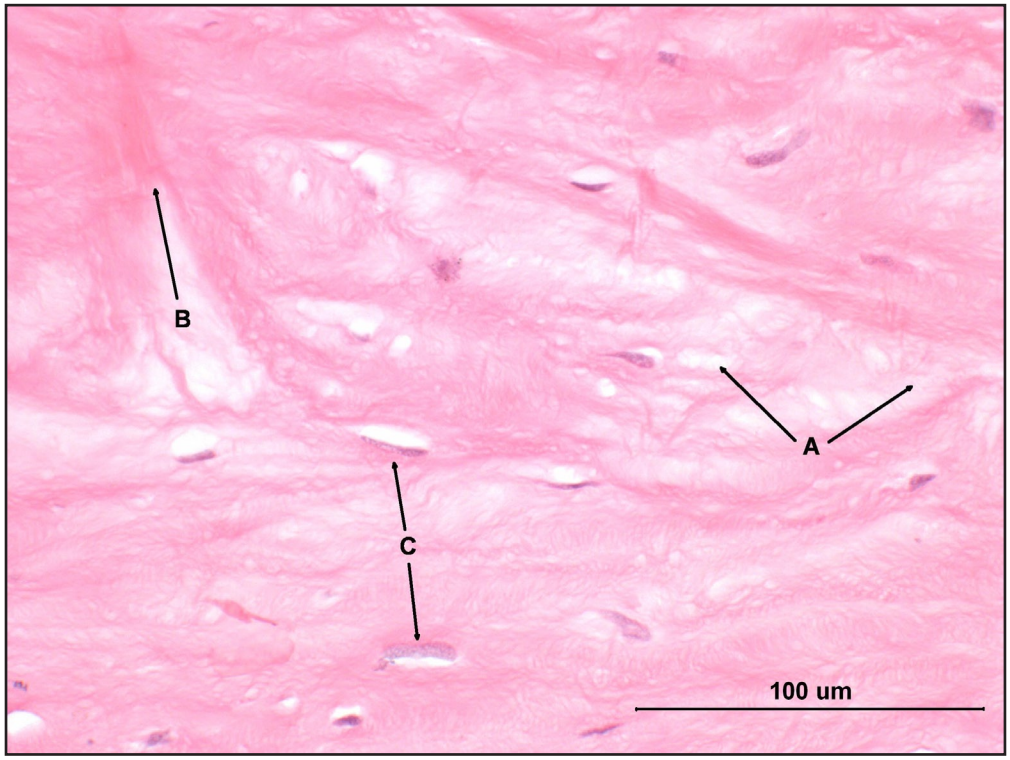

Figure 23. 06AG30, AA(10 minute exposure/120 minute post incubation) - Upper stroma with pyknotic keratocyte nuclei (⟶) (Slide C0979-1, 40x, H&E)

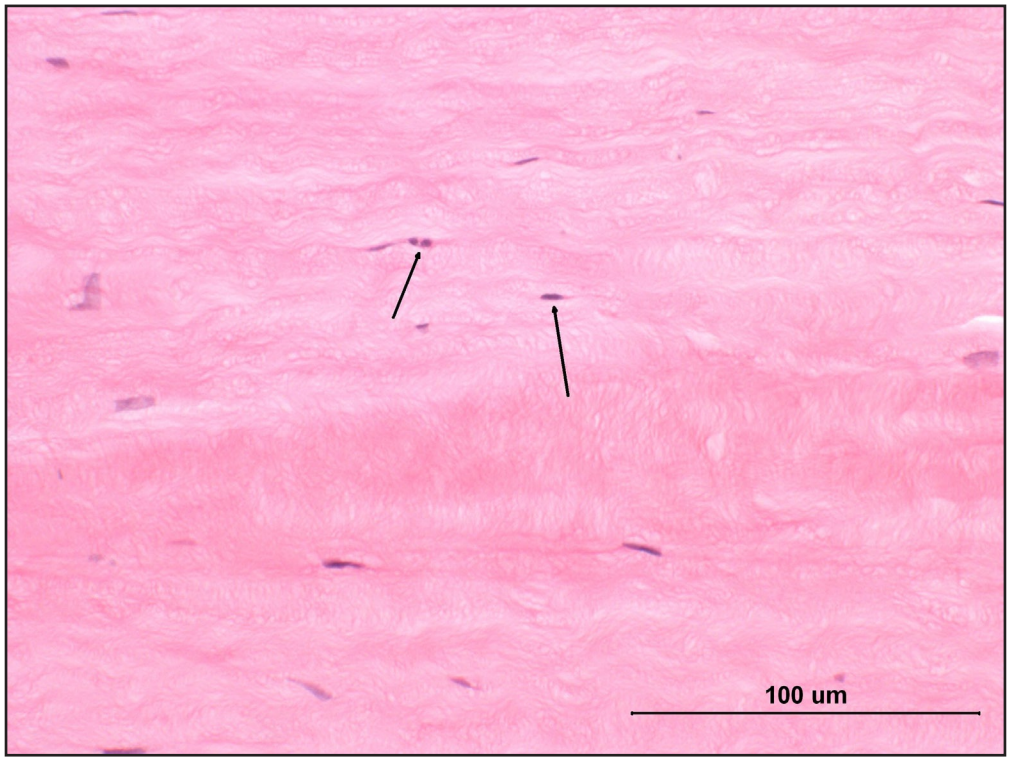

Figure 24. 06AG30, AA (10 minute exposure/120 minute post incubation) - Keratocytes in lower with eosinophilic cytoplasm (⟶) (Slide C0977-1, 40x, H&E)